



PRODUCT DESCRIPTION

FOR LAB RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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Protocol for Immunoblotting and Immunoprecipitation:

Preparation of Cell Lysate:

Add RIPA buffer to cells (100ul to a 35 mm dish, 200ul to a 60 mm dish, 500ul to a 100 mm dish) while the culture dish is placed on ice. Scrape the cells and gently rock the suspension on either a rocker or an orbital shaker in the cold room for 15 minutes to lyse cells. Sonicate in ice water with bath sonicator, until the sample is no longer viscous. Centrifuge the cells at 12000g at 4°C for 5 minutes to remove pellet. Move the supernatant to a fresh tube. Final concentration of cell lysate will be 2-3ug/ul. Add 5 x SDS stop buffer to the lysate to a 1xSDS final concentration.

Immunoprecipitation:

1. Prepare cell lysates or other protein samples. Cellular proteins or other protein samples should be labeled either metabolically or by iodination or biotination. For cell lysate, a concentration of 1-2ug/ul protein is optimum.
2. To 500 ug-1mg cell lysate, add 50ul of 50% slurry of protein A-sepharose beads and incubate the mix at 4°C for 15 minutes. Centrifuge at 10000g for 5 minutes at 4 °C. Collect the supernatant.
3. To the supernatant add 5ug of rabbit polyclonal antibodies against the specific antigen. Gently rock the reaction mixture for 1 hour at 4 °C.
4. Then add 50ul of 50% slurry of protein A-Sepharose beads to the reaction mixture and gently rock the sample at 4 °C for 30 minutes.
5. Spin the sample at 10000 g at 4°C for 3 minutes to pellet the beads.
6. Wash the beads twice with washing buffer A (10mM Tris-HCl, pH 8, 500mM NaCl, 0.5% NP-40 and 0.05% SDS), once with washing buffer B (10mM Tris-HCl, pH 8, 150mM NaCl, 0.5% NP-40, 0.05% SDS and 0.5% Dideoxycholate), once with washing buffer C (10mM Tris-HCl, pH 8, 0.05% SDS).
7. Then add 100ul of 1.25 x SDS loading buffer and boil the sample for 10 minutes before loading to SDS PAGE.
8. Detect the immunoprecipiated sample on SDS-PAGE either by direct radioautography or by further blotting biotin, depending on the initial labeling method.